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Biology of *Bordetella bronchiseptica*

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INTRODUCTION

Bordetella bronchiseptica has been recognized as a respiratory tract pathogen of mammals since 1910 (43). However, until the last decade most work with the genus *Bordetella* was with *Bordetella pertussis*, the causal agent of human pertussis (whooping cough). Although some research concerning the toxic, antigenic, and serological characteristics of *B. bronchiseptica* was reported by workers doing comparative studies of *B. bronchiseptica*, *Bordetella parapertussis*, and *B. pertussis*, little work was conducted on the etiological range and pathological nature of *B. bronchiseptica* in various animals until the last decade (34, 36, 40).

Increased interest in *B. bronchiseptica* has developed due to worldwide concern over respiratory diseases in confinement-reared swine, dogs, and laboratory animals. Since animal and economic losses from endemic respiratory diseases in these animals continue to increase, research teams, particularly in the economically advanced countries, have concentrated on determining the etiology and possible control of such respiratory diseases. Although diagnostic evaluations of infected animals repeatedly pointed to *B. bronchiseptica* as a primary etiological agent, early researchers had difficulty replicating similar laboratory-induced swine atrophic rhinitis, pneumonia, and canine infectious tracheobronchitis with pure cultures of *B. bronchiseptica* (123, 126). The lack of good laboratory models caused early investigators to conclude that *B. bronchiseptica* was only a secondary invader (3, 73, 87). Once Switzer (150) demonstrated laboratory-induced swine atrophic rhinitis (a respiratory infection causing atrophy of the nasal turbinates) with a pure culture of *B. bronchiseptica*, the primary role of *B. bronchiseptica* in swine became known. During the next decade the work of Switzer was verified by other researchers around the world (18, 24, 89, 106, 122, 122a, 142, 144, 160). In 1973 workers in Scotland demonstrated that *B. bronchiseptica* alone could cause acute infectious canine tracheobronchitis (kennel cough) (157, 166). Unlike infections in swine and dogs, the involvement of *B. bronchiseptica* as a primary pathogen of respiratory infections in laboratory animals was accepted much earlier (46, 47, 52, 59).

There was also a recent increase in interest in *B. bronchiseptica* by *B. pertussis* workers after Kloos ét al. (92) revealed that *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* have sufficient deoxyribonucleic acid (DNA) homology and similar enough bacteriological properties to be considered members of the same species. It is now believed that information concerning *B. bronchiseptica* may have a direct relationship to the understanding of *B. pertussis* (92). Furthermore, *B. bronchiseptica* now serves as a model for the study of both environmental effects on respiratory disease and plasmid biology. As no complete survey concerning *B. bronchiseptica* has been published, this review attempts to (i) summarize the bacteriological and pathological properties of *B. bronchiseptica*, (ii) survey the etiological role and immunological properties of this organism as related to mammalian

respiratory disease, and (iii) discuss the economic impact of this respiratory pathogen on humans.

CLASSIFICATION

B. bronchiseptica was first isolated and identified as *Bacillus bronchicanis* by Ferry in 1910 (43). The name *Bacillus bronchicanis* was chosen since the organism was isolated from the respiratory tracts of dogs suffering from distemper. After organisms with characteristics identical to those described by Ferry were isolated from the respiratory tracts of guinea pigs, monkeys, and humans in 1912 and 1913 (45, 146), the organism was renamed *Bacterium bronchisepticus* (128). In the ensuing years this bacterium was renamed four more times, as follows: *Alcaligenes bronchisepticus* Bergey (1925), *Brucella bronchiseptica* Topley and Wilson (1929), *Alcaligenes bronchicanis* Haupt (1935), and *Haemophilus bronchisepticus* Wilson and Miles (1946). It was placed in the genera *Alcaligenes*, *Brucella*, and *Haemophilus* due to morphological, growth, and biochemical characteristics which were somewhat similar to the characteristics of members of these genera. Finally, this bacterium was given its present name when Moreno-Lopez described the genus *Bordetella* (in honor of Jules Bordet, who first isolated the organism causing pertussis) and the species *Bordetella bronchiseptica* (128).

Biochemical and antigenic comparisons, DNA hybridization studies, and phage typing have indicated a close taxonomic relationship among the members of the genus *Bordetella*. These members (*B. bronchiseptica*, *B. pertussis*, and *B. parapertussis*) are minute coccobacilli which are nonmotile or motile by lateral peritrichous flagella, gram negative, and may stain bipolarly. They also possess respiratory metabolism, are nonfermentative, and produce no indole or hydrogen sulfide, but they do produce cytochrome oxidase, lysine decarboxylase, and catalase. They do not liquefy gelatin, but do render carbohydrates and litmus milk basic. Furthermore, all three species produce a blue protein called azurin, which undergoes reduction in the presence of concentrated cell-free extracts and succinate. Azurin is believed to act as a participant in the electron transport system between cytochrome *c* and cytochrome oxidase (149). The minimum nutritional requirements of the members of the genus *Bordetella* are very similar (Table 1), but they are quite different from those of organisms in the genera *Haemophilus* and *Brucella*. *Bordetella* species do not require X factor (hematin) or V factor (nicotinamide adenine dinucleotide) (129) and are distinguished

TABLE 1. *Minimum nutritional requirements of bordetella species*[a]

Substrate(s)	*B. pertussis*	*B. parapertussis*	*B. bronchiseptica*
Nicotinic acid	+	+	+
Glutamic acid, proline, leucine	−	−	+
Glutamic acid, proline, leucine, cystine, methionine	−	+	+
Glutamic acid, proline, leucine, cystine, methionine, alanine, asparagine, serine	+	+	+

[a] Substrates were added to ammonia basal salts medium. +, Growth turbidity in defined medium after five serial culture transfers; −, no visible growth turbidity.

by several phenotypic characters, such as rapid growth on blood-free peptone agar, motility, and various biochemical properties (Table 2) (17, 92, 128, 129, 154, 162). All *Bordetella* spp. are strictly aerobic, grow optimally at 35 to 37°C, and agglutinate erythrocytes from a variety of mammals and fowl (11, 58, 86, 91). The capacity of *B. bronchiseptica* to hemolyse blood is also used in identification. Beta-hemolytic colonies develop on agar media containing blood from a variety of mammals (11, 58, 86, 91, 100). However, differences in hemolytic response can be induced by varying the growth substrate. Pedersen grew *B. bronchiseptica* which produced strong hemolysis on solid media in an acid environment and only slight hemolysis in alkaline media (124). Furthermore, hemolytic substances considered to be terminal growth products have been found to consist of a heat-labile fraction and a heat-stable fraction (93). The three species of *Bordetella* are all pathogens of mammalian respiratory tracts; *B. pertussis* and *B. parapertussis* cause whooping cough in humans, and *B. bronchiseptica* causes respiratory infections in numerous other mammals.

The members of the genus *Bordetella* possess similar antigenic characteristics; each species possesses a genus-specific, heat-stable somatic (O) antigen, an antigenic heat-labile dermonecrotic toxin, and a common heat-labile agglutinogen (20, 35, 37, 48, 49, 84, 91). Furthermore, each species has a species-specific agglutinogen, and 10 other agglutinogens occur in all three species (128). Additional evidence for a close taxonomic relationship between *B. bronchiseptica* and *B. parapertussis* was reported by Rauch and Pickett, who used bacteriophage typing (131); 47% of the phage isolates from 48 *B. bronchiseptica* isolates were found to lyse nine heterologous strains of *B. parapertussis*. However, no analogous relationship was demon-

TABLE 2. *Distinguishing phenotypic characters of Bordetella species*[a]

Species	Growth on Bordet-Gengou agar	Growth on blood-free peptone agar		Motility	Citrate utilization	Urease activity	Nitrate reduction	Specific heat-labile antigen (factor)	Growth in presence of 20% citrate	Growth in presence of 10 mg of colloidal copper sulfide per liter
		Phase I	Phase IV							
B. pertussis	Slow, small colonies	−	+	−	−	−	−	1	−	+
B. parapertussis	Moderate, slightly larger colonies	+	With browning	−	±, +	+	−	14	−	−
B. bronchiseptica	Moderate to rapid, larger colonies	+	+	+	+	+ (Rapid)	+	12	+	+

[a] +, Positive; ±, weak positive; −, negative.

strated in similar tests conducted on 50 strains of *B. pertussis*. Further evidence was reported by Kloos et al. (92), who conducted DNA-DNA reassociation tests on the members of the genus *Bordetella*. Relative binding values for individual single-strand DNAs were 72 to 93% between *B. bronchiseptica* and *B. pertussis* and 88 to 94% between *B. pertussis* and *B. parapertussis*. Also, leucine and tryptophan autotrophs of *B. pertussis* strains were transformed by *B. bronchiseptica* and *B. parapertussis* DNAs at frequencies close to homologous DNA strand reassociation values. A numerical taxonomic survey was conducted by Johnson and Sneath (85), who found an average intergroup similarity of approximately 80% between *B. bronchiseptica* and the other two *Bordetella* species. Like *B. pertussis*, *B. bronchiseptica* has been shown to produce a biologically active component which provides histamine sensitization (25). The results of these comparative studies question the validity of classifying these three organisms as separate species. The taxonomic placement of the genus *Bordetella* still is undecided. In *Bergey's Manual of Determinative Bacteriology*, 8th ed. (128), this genus is placed in the gram-negative aerobic rods and cocci section under genera of uncertain affiliation, along with *Alcaligenes*, *Acetobacter*, *Brucella*, *Francisella*, and *Thermus*.

DESCRIPTION OF *B. BRONCHISEPTICA*

Morphology

B. bronchiseptica is readily identified as a gram-negative, nonsporeforming, pleomorphic, coccobacillary bacterium. Cells grown on solid media occur mainly in coccoid form, often ranging in size from 3.0 by 0.5 μm to 0.4 by 0.72 μm (Fig. 1) to 0.5 by 0.4 μm; some filamentous forms have an average size of 0.4 by 8.0 μm (133) (Fig. 2). Unlike *B. pertussis* and *B. parapertussis*, *B. bronchiseptica* is motile. Motility is provided by peritrichous flagella. Electron micrographic

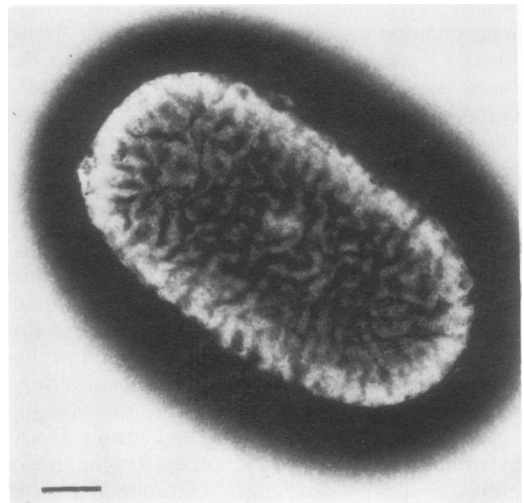

FIG. 1. *Electron micrograph of a negatively stained B. bronchiseptica cell. Blood agar culture (48 h); bar = 0.1 μm. (Reproduced with permission from D. O. Farrington [D. O. Farrington, Ph.D. thesis, Iowa State University, Ames, 1974].)*

studies have shown flagella which resemble a left-handed triple helix and have an average diameter of 13.9 nm (95). Richter and Kress (133) photographed multistranded flagella of *B. bronchiseptica* which were 18 to 22 nm thick and contained braided structures consisting of five to six strands per flagellum; each strand was 2 nm wide. These flagella resembled those of other gram-negative bacilli.

Workers have also conducted fine structure evaluations, such as outer membrane protein identity determinations and plasmid characterizations, to define isolate characteristics. Fine structure studies on *B. bronchiseptica* have shown that the cell wall and the membranes are similar to those of other gram-negative bacteria. This organism possesses a cell wall composed of five layers. The outer three layers give the surface contours a lobulated appearance, with chan-

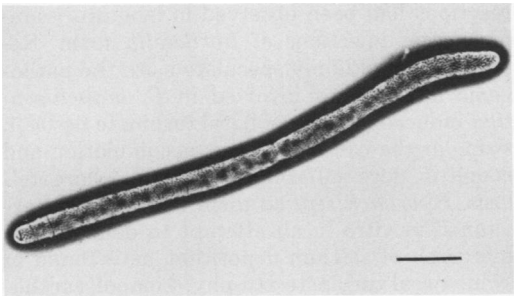

FIG. 2. *Electron micrograph of a negatively stained B. bronchiseptica cell. Tryptose phosphate broth culture (24 h); bar = 1.0 μm. (Reproduced with permission from D. O. Farrington [Ph.D. thesis].)*

nels 10 to 20 nm wide between lobules (133) (Fig. 3). The outer membrane proteins of four smooth and two rough strains of *B. bronchiseptica* were analyzed and compared with the outer membrane proteins of phase I and IV isolates of *B. pertussis* (26). Smooth strain *B. bronchiseptica* isolates provided profiles similar to those of *B. pertussis* with the following two exceptions: proteins from two of the smooth strains of *B. pertussis*, which had molecular weights of 98,000 and 88,000, were not present in the smooth strains of *B. bronchiseptica*. The rough strains of *B. bronchiseptica* resembled phase IV *B. pertussis* isolates in that outer membrane proteins of 27.5 and 30 kilodaltons were missing in the rough phase of both species. This study suggests that the control mechanisms exerted over the production of these outer membrane proteins are similar in these two *Bordetella* species. Richter and Kress (133) found that the cell membrane of *B. bronchiseptica* is trilaminar and surrounds cytoplasm which contains abundant ribosomes in a cytoplasmic matrix. The nuclear zones of *B. bronchiseptica* consist of networks of fibrils thought to be DNA and dense undefined bodies (Fig. 3). Plasmid bodies have been observed in various *B. bronchiseptica* isolates. Terakado et al. (155) first inferred the presence of plasmids from R-factor studies with drug-resistant strains of *B. bronchiseptica* isolated from swine. Later, Dobrogosz et al. (26) screened seven *B. bronchiseptica* strains for the presence of plasmid DNA. They found that four of the seven strains carried one or more medium to large plasmids in addition to a small labile plasmid similar in size and concentration to a plasmid found in *B. pertussis* and *B. parapertussis*.

Although *B. bronchiseptica* can usually be characterized, proper descriptions and comparisons of *B. bronchiseptica* isolates have been hindered by the lack of a universally accepted descriptive system. Scientists commonly describe gross colony morphology, colony phase typing, and animal infectivity tests in an attempt to distinguish among such isolates.

When *B. bronchiseptica* isolates are grown on agar, colony morphologies range from smooth to rough. Early researchers designated smooth phase I colonies as virulent and rough phase IV colonies as avirulent. Disagreement with this system developed when more recent investigations (101, 121) revealed multiple intermediate colony phase types (phases II and III). Nakase (116, 117) found that *H. bronchisepticus* (*B. bronchiseptica*) phase I (smooth) colonies were pathogenic for mice, whereas phase IV (rough) colonies were avirulent. Phase I colonies were found to be extremely unstable and to be transformed readily to phase II, III, and IV colonies after several growth passages on artificial media. Further evidence of phase instability was reported by Lacey (96), who observed more phase I colonies on Bordet-Gengou and blood agars than on nutrient agar. These observations suggest that the composition of the growth medium may affect the colonial phase type. Differences within phase III (phases III-1 and III-2) were found to be associated with specific capsular antigens or somatic antigens or both. Rough phase IV cells have been considered to be phase III cells lacking only flagellar antigens (116, 117). While attempting to detect enzymatic differences between phase types, Endoh et al. (38) discovered an abundance of adenylate cyclase activity in phase I cultures of all three *Bordetella* species. *B. parapertussis* and *B. bronchiseptica* released this enzyme to the culture fluid, whereas *B. pertussis* produced more activity

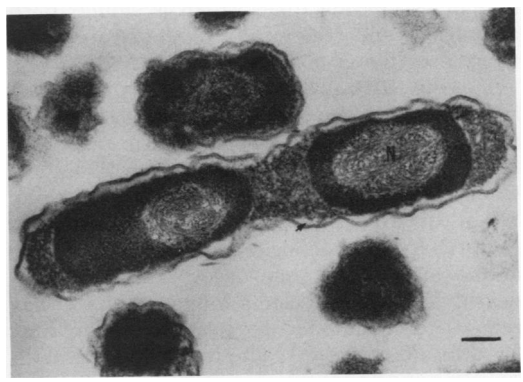

FIG. 3. *Longitudinal cross-section of B. bronchiseptica. Tryptose phosphate broth culture (24 h); uranyl acetate and lead citrate stain. Note the furrowed cell wall. The nucleoplasms (N) appear to be whorled and rarefied. The retraction of the bacterial cell membrane and cytoplasm from the cell wall is evident (arrow). Bar = 0.1 μm. (Reproduced with permission from D. O. Farrington [Ph.D. thesis].)*

intracellularly. Phase III cultures of these species lacked both extracellular and intracellular enzyme activity.

Inconsistencies in descriptions of phase types with respect to virulence, motility structures, and ability to attach to membrane surfaces have been reported. For example, Nakase noted peritrichous flagella on smooth phase I cells and no flagella on rough phase IV cells, whereas Bemis et al. (11) observed nonflagellated phase I cells and flagellated phase IV cells. The smooth-virulent and rough-avirulent character designations used successfully to categorize *B. pertussis* have not held true at my laboratory in virulence designations of *B. bronchiseptica* isolates. Smooth, intermediate, and rough colony forms of *B. bronchiseptica* have demonstrated similar virulence in causing swine and canine respiratory infections. Similarly, other investigators (B. Plotkin and D. Bemis, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, B54, p. 26) have found that smooth-, intermediate-, and rough-phase *B. bronchiseptica* isolates demonstrate host cell infectivity by attachment to hamster lung fibroblasts. In contrast, *B. bronchiseptica* phase I isolates have been shown to adhere both to swine nasal epithelial cells cultured in vitro and to nasal epithelium in experimentally infected pigs, whereas only a few phase III isolates have displayed similar attachment to either type of cells (170). Obviously, more investigation is needed in this area before a colony phase typing system for *B. bronchiseptica* isolates can be considered meaningful.

Toxins

All *Bordetella* species synthesize at least one lipopolysaccharide toxin which is similar both chemically and biologically to the lipopolysaccharide toxins of other gram-negative bacteria (20, 34, 40, 104). A heat-labile toxin(s) prepared from freeze-thawed extracts of *B. bronchiseptica* and *B. pertussis* proved to be toxic to guinea pigs and mice and caused dermonecrotic lesions when injected subcutaneously into rabbits. When this toxin was injected intravenously into rabbits, hyperglycemia was induced initially, and later hypoglycemia occurred, resulting in death. The hypoglycemia-inducing factor was reduced or eliminated by heating the toxin at 55°C for 30 min (118). The toxin was Formalin sensitive and nonantigenic and lost potency after filtration through a Seitz E-K filter. In contrast, Evans (39) found that formalized *B. bronchiseptica* toxin was antigenic and that the resulting antitoxin prepared against *B. bronchiseptica* toxin neutralized the toxins of all three *Bordetella* species.

Dermonecrotic lesions and adverse systemic reactions had been observed in laboratory animals after injections of *Bordetella* toxin. Researchers could only speculate as to the pathogenic mechanisms involved in *B. bronchiseptica*-induced atrophy of nasal turbinate tissue in swine or the cause of mucus accumulation and cough in dogs suffering from canine bordetellosis. *B. bronchiseptica* toxin extracts were examined in vitro in an attempt to demonstrate inhibition of calcium deposition, as is found in swine nasal turbinate atrophy. Lipopolysaccharides extracted with trichloroacetic acid from *B. bronchiseptica* and *B. pertussis* were shown to affect markedly respiration, certain energized processes, and the morphology of beef heart mitochondria. The interaction of this endotoxin-like extract with intact mitochondria inhibited the coupling of phosphorylation to terminal electron transfer and Ca^{2+} translocation (76). Furthermore, electron microscopic studies demonstrated that *B. bronchiseptica* endotoxin exists in the form of protein-lipopolysaccharide complex vesicular membranes. These endotoxin membranes appear to interact with mitochondrial membranes and to modify mitochondrial enzymatic processes and morphology (76). More recently, Nakase conducted in vivo studies with swine, using a sonicated cell-free toxin extract of phase I *B. bronchiseptica* cells, which was inoculated intranasally into young pigs each day for 64 days. Later, varying degrees of nasal turbinate atrophy were observed in treated pigs (75). This extract was then fractionated into an endotoxin component and a heat-labile dermonecrotic toxin component. No nasal turbinate atrophy was produced in young mice by the endotoxin fraction, whereas severe atrophy was produced by the heat-labile dermonecrotic toxin fraction (Y. Nakase, K. Kume, K. Shimoda, and A. Sawata, Proc. Int. Pig Vet. Soc. Congr., p. 202, 1980). This work questions the concept proposed by Harris et al. and others (79, 80) that *B. bronchiseptica* releases a toxic factor(s) which alters normal formation of the bony nasal turbinates to the extent that damage may be caused by the heat-labile dermonecrotic toxin rather than by the endotoxin. Other toxic factors are also produced by *B. bronchiseptica*. Dixon et al. (25) have shown that intravenous administration of histamine to dogs injected with *B. bronchiseptica* produces a significant increase in the rate of discharge of lung irritant receptors and reduces airflow through the respiratory tract. This study suggests that a histamine-sensitizing factor is produced by *B. bronchiseptica*. However, in tests of the *Bordetella* species, Ross et al. found a histamine-sensitizing factor only in *B. pertussis* (135). Further investigations concerning the physical properties and bioactivities

of the various *B. bronchiseptica* toxins are needed.

Antigenic Factors

The following three types of agglutinogens from *B. bronchiseptica* have been described: flagellar H antigens, surface heat-labile K antigens, and surface heat-stable O antigens (1, 35, 36, 99). Although nonmotile *B. pertussis* and *B. parapertussis* lack H antigens, all three *Bordetella* species possess similar O antigens and antigenic, thermolabile, hemorrhagic toxin antigens (37). Each species demonstrates a different major K antigen, whereas the strains within each species exhibit varying numbers of K partial antigens (1). A total of 14 K antigens have been shown to be shared by the three *Bordetella* species (128).

In an extensive study, Nakase (116) attempted to clarify further the antigenic structure of *H. bronchisepticus* (*B. bronchiseptica*) isolates. Differences between morphological and antigenic structures and the changes in these structures due to colonial phase shifts were measured with monospecific sera. The results indicated that *H. bronchisepticus* could be divided into three smooth phases and one rough phase. Smooth phase I and II cells possessed identical L (heat-labile capsular), H, S (thermostabile surface), O1, O5, and O7 antigens. Phase III cells were divided antigenically into phase III-1 (with H, O1, and O7 antigens) and phase III-2 (with H, O5, and O7 antigens). Rough phase IV cells possessed only O1 and O5 antigens.

The antigenic properties of *Bordetella* are affected by the type of nutrients used for growth and by the number of serial passages made on laboratory growth media. Both *Haemophilus pertussis* (*B. pertussis*) and *H. bronchisepticus* antigens showed altered serological responses when cultivated on a medium in which $MgSO_4$ replaced NaCl (96, 97). Flosdorf et al. (53) verified that an antigenic difference between the agglutinabilities of freshly isolated and laboratory-stored *H. bronchisepticus* strains could be detected. Laboratory strains failed to agglutinate in sera prepared against freshly isolated strains. Although the surface antigens of *B. bronchiseptica* have been well characterized, the antigenic spectra of the attachment structures (pili), which are being studied now in several laboratories, have yet to be documented.

Antibacterial Susceptibilities

B. bronchiseptica is susceptible to various antimicrobial agents both in vivo and in vitro (6, 55, 152). However, there have been problems in providing and maintaining effective concentrations of such agents on infected membrane surfaces without causing unwanted residues in tissues, particularly in animals used for producing human food. An equally difficult problem arising from the use of such compounds is the development of resistant strains of *B. bronchiseptica*.

Primary *B. bronchiseptica* isolates have been reported to be highly resistant to a wide spectrum of antibacterial agents (143, 151, 152). Switzer and Farrington (152) reported that *B. bronchiseptica* was susceptible to the sulfonamide drugs, in particular sulfamethazine and sulfathiazole. Initially, these drugs were effective in controlling *B. bronchiseptica* infections in swine herds; however, later investigations proved that resistant strains had developed. Harris and Switzer (77) reported that 85% of the *B. bronchiseptica* isolates recovered from pigs in 25 herds were resistant to sulfonamides. Another survey involved 61 swine isolates which were tested for susceptibility to 52 antibacterial drugs; 11% of the isolates were resistant to sulfa drugs and cross-resistant to aminobenzyl penicillin and streptomycin (6). Wilkins and Helland (164) also found that *B. bronchiseptica* isolates recovered from dogs with tracheobronchitis were resistant to lincomycin, penicillin, streptomycin, nitrofurantoin, and tylosin. In contrast, many of these isolates from dogs were susceptible to novobiocin, tetracycline, ampicillin, chloramphenicol, erythromycin, and kanamycin (164).

More recently, Bemis and Appel (8) found that *B. bronchiseptica* isolates were susceptible to chlorhexidine (Nolvasan) when evaluated in vitro, whereas Nolvasan was of no value in reducing the number of *B. bronchiseptica* cells in the respiratory tracts of infected dogs. In additional studies, seven widely used antibiotics were administered by oral, parenteral, and aerosol routes to dogs before aerosol instillation of virulent *B. bronchiseptica*. Parenterally and orally delivered antibiotics caused no reduction in bacterial numbers in the tracheae or bronchi of infected dogs. Aerosolized antibiotics did reduce the bacterial populations from similar tissues and the clinical signs of infection for up to 3 days posttreatment (9). Turkey poults intranasally injected with *B. bronchiseptica* were treated with tetracycline hydrochloride or sulfaquinoxaline. Tetracycline-treated birds showed significant reductions in the level of infection; in contrast, sulfaquinoxaline-treated birds showed only slight improvement (61).

Eventual failure of many antimicrobial agents to control swine respiratory disease has prompted investigators to determine whether resistance to these agents is mediated by R factors carried by *B. bronchiseptica*. Hedges et al. (81) derived a 34.6-megadalton plasmid from

a wild strain of *B. bronchiseptica*; this plasmid conferred resistance to ampicillin, streptomycin, sulfonamides, and mercury salts. Terakado et al. (156) also demonstrated that R factors carrying sulfadimethoxine, streptomycin, and aminobenzyl penicillin resistance were present in strains of *B. bronchiseptica* isolated from pigs. All strains could transfer drug resistance as one unit to a susceptible strain of *Escherichia coli*, as well as to other *B. bronchiseptica* strains. Terakado et al. (155) later demonstrated that, unlike the R factors from *Enterobacteriaceae* and *Pseudomonas aeruginosa* strains, the R factors from *B. bronchiseptica* could be characterized by the lack of tetracycline resistance but the presence of aminobenzyl penicillin resistance. Yaginuma et al. (167) selected an R plasmid from a *B. bronchiseptica* isolate and demonstrated the production of a β-lactamase which was highly active against phenethicillin, oxacillin, and propicillin. Furthermore, Terakado et al. (155) conducted a survey of *B. bronchiseptica* isolates for drug resistance and the distribution of R factor; they found that 71 of 304 strains (23%) were resistant to either one or more of the drugs tested (streptomycin, sulfadimethoxine, and aminobenzyl penicillin). Many of these 71 strains contained triple resistance, and 86% of the 71 strains carried R factors capable of conjugal transfer. Since *B. bronchiseptica* readily transfers multitype resistance to a wide variety of antimicrobial substances, researchers have tried to counter this problem with such alternative techniques as the development of vaccines for controlling bordetellosis.

Isolation and Cultivation

B. bronchiseptica can be recovered readily from the respiratory tracts of animals with bordetellosis by nasal or tracheal swabbing. Primary swab isolates are usually plated onto selective media containing penicillin, streptomycin, and nystatin (a fungus inhibitor) (56). Other types of selective media have also been used successfully (41, 56, 57).

Usually, plates are incubated at 35 to 37°C for 40 to 72 h, and the resulting colonies are observed for pearlescent, smooth-margin appearance. Cells from presumptive colonies are Gram stained, and then similar colonies are tested for genus- and species-specific biochemical reactions. *B. bronchiseptica* is often differentiated from other gram-negative coccobacilli by using 200 μg of nitrofurantoin per ml of plating medium (57). *B. bronchiseptica* can be propagated both on agar surfaces and in broth cultures containing standard protein media (e.g., tryptose phosphate and Trypticase soy media). For maximum growth in broth, aeration, temperatures of 35 to 37°C, and control of released alkaline factors with standard buffer salts are necessary (Goodnow, unpublished data).

The identification of primary *B. bronchiseptica* isolates has been reported to be more difficult than the identification of laboratory-maintained strains. When grown on blood agar, laboratory-maintained *B. bronchiseptica* colonies are usually hemolytic and glistening and develop an average diameter of 2.0 mm in 1 to 2 days. However, Simpson and Simmons (145) reported isolating primary cultures from rodent nasopharyngeal swabs which produced uncharacteristic, nonhemolytic colonies with diameters of 0.1 to 0.2 mm after 24 h of incubation. Furthermore, the motility of these isolates was often undetected in cultures that were not incubated for more than 3 days.

DISEASES

B. bronchiseptica has been associated with respiratory diseases of numerous mammals since it was first reported in 1910 (43, 44, 169). This organism has been isolated from dogs (43), humans (19, 45, 60), monkeys (71, 138), cats (52, 147), rabbits, ferrets, guinea pigs (111), mice (88), swine (32), foxes (134), rats (16, 153), hedgehogs (33), horses (58, 107), skunks, opossums, raccoons (153), koala bears (112), turkeys (51, 83), and lesser bushbabies (94). Humans are not usually considered to be natural hosts for *B. bronchiseptica*; however, six cases of human bordetellosis have been reported since 1910 (19, 23, 46, 60, 111, 165). Since the greatest economic losses from bordetellosis have been incurred from infected swine, dogs, and laboratory animals, most investigations have been conducted with these three animal groups.

Swine Atrophic Rhinitis and Pneumonia

Franque (54) first reported atrophic rhinitis of swine as a condition in which affected swine did not fatten, developed atrophy of the nasal and ethmoid turbinates, and, in severe cases, developed malformation of the nose. Early in the study of this disease, the condition was thought to be linked to nutritional or genetic deficiencies (54, 82, 130). However, since the 1950s the possible nutritional and genetic nature of this disease has been generally discounted (103, 152). Infectious agents were then considered, and finally Switzer (150) reported that *B. bronchiseptica* recovered from atrophic swine turbinates produced turbinate atrophy when it was instilled intranasally into newborn pigs. This work, which was later confirmed, demonstrated that *B. bronchiseptica* is a primary pathogen of swine (18, 24, 62, 76, 89, 106, 122, 122a, 136). Recently, using gnotobiotic pigs, Martineau et al. (B. Mar-

tineau, M. Josse, B. Martineau-Doize, and F. Coignoul, Proc. Int. Pig Vet. Soc. Congr., p. 201, 1980) determined that the minimum infective dose for *B. bronchiseptica*-induced atrophic rhinitis is 3.0×10^5 cells per ml when the organism is inoculated intranasally (0.5 ml/nostril) for 3 consecutive days. Shortly after the report of Switzer, the biological activity of *B. bronchiseptica* on upper respiratory tract tissue of infected swine was assessed. Duncan et al. (28, 30) found *B. bronchiseptica* cells on epithelium membranes of swine turbinates and tracheae by using fluorescent antibody staining. The organism did not appear to invade underlying tissue. A further examination showed that *B. bronchiseptica* caused nasal mucus membrane cilia to swell to a polyhedral shape, to be fewer in number, and to become abnormally spaced. An evaluation of the osteoblasts and osteocytes of bone cells from pigs infected with *B. bronchiseptica* also showed swollen mitochondria, distention of cisternae of endoplasmic reticula, and some lysis of cells (50).

Variation in the pathogenicities of *B. bronchiseptica* isolates for pigs has been reported. Intranasal inoculations of pigs with swine, rabbit, cat, and rat isolates caused mild to moderate turbinate atrophy, whereas an isolate of dog origin caused no turbinate hypoplasia (137). Furthermore, *B. bronchiseptica* variants have been isolated from the nasal secretions of swine considered to be free from clinical respiratory disease (102), indicating that these variants may colonize a host but not produce disease symptoms. More recently, Skelly et al. (B. J. Skelly, M. Pruss, R. Pellegrino, D. Andersen, and G. Abruzzo, Proc. Int. Pig Vet. Soc. Congr., p. 210, 1980) reported that numerous *B. bronchiseptica* field isolates recovered from swine could infect the nasal turbinates of other susceptible swine. However, only 50% of these isolates produced a significant degree of nasal turbinate atrophy in the inoculated swine.

B. bronchiseptica has also been reported to cause swine pneumonia (4, 27, 29, 32, 69, 70, 86, 148, 159). Phillips (127) considered *B. bronchiseptica* to be a primary agent in Canadian swine suffering from pneumonia. In contrast, Betts (15) recovered *B. bronchiseptica* from pneumonic lungs of pigs raised in England, yet considered the organism to be a secondary invader, following virus pneumonia in pigs. Ray (132) found *B. bronchiseptica* associated with pneumonia in swine in the United States and suggested the term porcine whooping cough for swine pneumonia. Swine pneumonia has been induced readily in controlled laboratory experiments. L'Ecuyer et al. (100) reproduced pneumonia in swine by using a combination of intratracheal and intranasal inoculations of *B. bronchiseptica* grown in embryonated chicken eggs. Meyer and Beamer (113) verified this work by inoculating germfree swine with *B. bronchiseptica* and inducing experimental pneumonia.

Canine and Feline Bordetellosis

Ferry (43) repeatedly isolated *Bacillus bronchicanis* (*B. bronchiseptica*) from ocular, nasal, and tracheal tissues of dogs suffering from distemper. At that time the etiology of canine distemper had not been defined clearly, although Carré (22, 22a) had reported that canine distemper was caused by a virus. Ferry reported inducing distemper-like symptoms (nasal rhinitis, bronchitis with persistent cough, bronchopneumonia, vomiting, blood diarrhea, and conjunctivitis) by blowing dried *Bacillus bronchicanis* (*B. bronchiseptica*) into the nasal passages of dogs, guinea pigs, rabbits, and monkeys. He concluded that *Bacillus bronchicanis* (*B. bronchiseptica*) was the primary etiological agent causing canine distemper. Later, however, Dunkin and Laidlaw (31, 98) and Torrey and Rahe (161) demonstrated conclusively that canine distemper was in fact caused by a virus. Apparently the test dogs used by Ferry were suffering concurrently from bordetellosis and distemper. Even after the etiology of canine distemper and control measures for this disease were defined, other respiratory infections continued to persist in dogs. Veterinarians worldwide are still often confronted with dogs suffering from infectious tracheobronchitis (canine cough).

Canine infectious tracheobronchitis is considered to be a highly contagious respiratory tract disease which affects dogs of all ages. Severely affected dogs often have a dry, harsh, hacking cough, followed by retching and vomiting. The syndrome usually lasts for 1 to 3 weeks, and occasionally pneumonia-related deaths occur. The transmission and frequency of this disease are highest among dogs kept under close confinement, as in breeding and boarding kennels. Kennel cough has long been considered to be a disease complex having viral and bacterial components (2). Although Pennock and Archibald (126) attributed canine tracheobronchitis to *Brucella* (*Bordetella*) *bronchiseptica* infections, another investigator reported inconsistent results when he attempted to induce clinical tracheobronchitis by intranasal inoculation with *B. bronchiseptica* (73). Once Wright et al. (166) discovered that a pure culture of *B. bronchiseptica* could be utilized as an aerosol inoculum to induce disease in dogs, investigators easily demonstrated laboratory-induced infectious tracheobronchitis and pneumonia (7, 9, 12, 157). In addition, *B. bronchiseptica* was found to cause

naturally occurring respiratory infections in kennels (10, 159). It has now been demonstrated that the most severe symptoms of the disease (excessive tracheal mucus accumulation, vomiting, weight loss, and pulmonary lesions) are linked to *B. bronchiseptica* (2, 12, 67). Similar symptoms are not obtained when dogs are inoculated with only canine respiratory viruses.

B. bronchiseptica has been reported infrequently as a respiratory pathogen in cats. Fisk and Soave (52) reported that about 10% of the cats which they sampled either were carriers of *B. bronchiseptica* or suffered from *B. bronchiseptica*-caused pneumonia. Snyder et al. (147) reported that 10% of 127 cats suffering from respiratory tract disease were infected with *B. bronchiseptica*. The incidence and severity of feline bordetellosis need to be assessed further.

Respiratory Infections of Laboratory Animals

The laboratory animal industry, which provides rabbits, guinea pigs, rats, monkeys, ferrets, and other mammals to the scientific research community, has long labored against animal losses from epizootic respiratory infections. *B. bronchiseptica* has often been cited as a primary etiological agent in many of these outbreaks. Ferry (45, 46) isolated *Bacillus bronchisepticus* (*B. bronchiseptica*) from rabbits, guinea pigs, ferrets, and monkeys affected with an epizootic respiratory infection. Later, Ferry and Hoskins (47) and Oldenburg et al. (119) reported that the majority of rabbit catarrh cases, which were characterized by nasal discharge, sneezing, loss of appetite, and weight loss, were caused by *Bacillus bronchisepticus*. Other workers induced ventral turbinate atrophy in newborn rabbits with intranasal inoculations of *Alcaligenes bronchisepticus* (*B. bronchiseptica*) (105, 106). Germfree and conventional weanling rats and mice were exposed to *B. bronchiseptica* and later developed acute to subacute bronchopneumonia (21, 165). *B. bronchiseptica* has also been reported to be associated with otitis media of guinea pigs (163).

Research in which primates are used as test animals is both costly and highly regulated by various agencies. Undesired respiratory epizootics among these animals are always a major concern. *B. bronchiseptica* has repeatedly been shown to be associated with pneumonia in primates (63, 71, 72). Graves (72) isolated *B. bronchiseptica* from laboratory-housed monkeys which were suffering from epizootic pneumonia. Seibold et al. (138) reported that 27% of bronchopneumonia cases were associated with *B. bronchiseptica* in *Calicebus* species. Currently, intranasal live *B. bronchiseptica* vaccines to control bordetellosis outbreaks are being evaluated in primates.

Human Infections

B. bronchiseptica is seldom considered to be infectious for humans. However, McGowan (111) isolated *Bacterium bronchicanis* (*B. bronchiseptica*) from 1 of 13 laboratory animal caretakers. Ferry (46) isolated *Bacterium bronchicanis* from an animal caretaker suffering from grippe. Winsser (165) also isolated *B. bronchiseptica* from 1 of 23 animal caretakers. The one caretaker positive for *B. bronchiseptica* had prior bronchopneumonia with a recurrent croup-like, nonproductive cough resembling a mild paroxysm of whooping cough. Furthermore, Brown (19) reported isolating *Bacillus bronchisepticus* from a child with symptoms of pertussis. It was believed that the child contacted the organism by handling a pet rabbit suffering from contagious nasal catarrh (snuffles). Switzer et al. (153) collected nasal swabs from 80 people who had had close contact with *B. bronchiseptica*-infected swine. However, no *B. bronchiseptica* was isolated from any of the humans sampled. Chang et al. (23) reported that *B. bronchiseptica* was responsible for posttraumatic purulent meningitis in a 9-year-old boy who had been kicked in the face by a horse. More recently, Ghosh and Tranter (60) cited a case of fatal bronchopneumonia in a malnourished alcoholic in which *B. bronchiseptica* was isolated from the blood and tracheal pus. These few reported cases of human *B. bronchiseptica*-related infections suggest that *B. bronchiseptica* may be capable of occasionally infecting humans under atypical conditions.

IMMUNITY

Prevention of *B. bronchiseptica* respiratory tract (lung, tracheal, or nasal turbinate) membrane infections of mammals appears to be dependent upon prevention of attachment to and colonization of host cells by invading bacteria. As there have been few reports of septicemic-phase infections, the inhibition of these infections appears to be dependent upon localized activity of humoral agglutinins, antitoxins, or cellular immune factors. Natural resistance in guinea pigs to both nasal reinfection and tracheal reinfection by *B. bronchiseptica* after an initial infection was reported by Yoda et al. (168). Numerous attempts to simulate this resistance with vaccines have been made. Prepared *B. pertussis* and *B. bronchiseptica* antigens have been administered in a variety of forms, with and without adjuvants and by various routes of administration. Cross-immunity studies have also been conducted by using *Bordetella* antigens (78, 90). Recently, subunit or

avirulent live *B. bronchiseptica* vaccines have been used to immunize swine, guinea pigs, and dogs in attempts to improve immune responses to bordetellosis compared with the responses which are usually induced by inactivated whole-cell bacterins.

Whole-cell, chemically inactivated *B. bronchiseptica* bacterins containing either Freund incomplete or aluminum hydroxide adjuvant have been used successfully to immunize mice and guinea pigs against death, the carrier state, and bronchopneumonia resulting from laboratory-induced or natural *B. bronchiseptica* infections (57, 59, 102). However, in some cases complete clearance of infecting *B. bronchiseptica* cells from nasal secretions has not occurred until months after vaccination. Goodnow et al. (66) inoculated mice intraperitoneally with both a *B. bronchiseptica* bacterin not containing adjuvant and a *B. bronchiseptica* bacterin containing aluminum hydroxide adjuvant and were able to prevent death in intraperitoneally challenged mice. Similarly, dogs and swine have been inoculated with whole-cell *B. bronchiseptica* bacterins with and without adjuvants in attempts to control bordetellosis. McCandlish et al. (108, 110) inoculated dogs with inactivated whole-cell bacterins with and without aluminum hydroxide adjuvant. Dogs inoculated with a bacterin not containing adjuvant coughed for a shorter time than control dogs, whereas dogs vaccinated with a bacterin containing adjuvant demonstrated significant protection against clinical canine bordetellosis. In another study (109), a heat-inactivated *B. bronchiseptica* bacterin containing no adjuvant was tested. Both vaccinated and control dogs developed clinical respiratory disease after aerosol challenge with *B. bronchiseptica*. However, the onset of disease in the vaccinated animals was delayed, thus indicating the necessity of using an adjuvant with the antigen for optimum immunization. Shelton et al. (140) utilized a *B. bronchiseptica* whole-cell bacterin containing aluminum hydroxide adjuvant and reported a reduction in clinical signs and pulmonary lesions in dogs reared in a closed colony undergoing a *B. bronchiseptica* epizootic.

Inactivated *B. bronchiseptica* bacterins have provided only marginal protection against canine bordetellosis induced in laboratories and have proven to be unsafe for use. The use of such bacterins has resulted in swelling and abscess formation at the inoculation site, as well as lameness in a significant number of vaccinated dogs (139). However, the use of similar bacterins in swine to control infectious atrophic rhinitis has been more successful. Infected swine often display such snout and facial distortions that their value is reduced severely. Furthermore, these infections often cause pigs to gain weight at a reduced rate, resulting in an extended rearing time, tying up rearing facilities, and, ultimately, ineffective swine production. *B. bronchiseptica* bacterins have been utilized in attempts to prevent snout distortion and nasal turbinate atrophy and, particularly, to accelerate nasal membrane clearance of *B. bronchiseptica*. Even though earlier workers were able to immunize guinea pigs against *B. bronchiseptica* infections (59, 115, 165), Harris and Switzer (77) could not induce nasal resistance against *B. bronchiseptica* infection by vaccinating swine intramuscularly with a whole-cell bacterin not containing adjuvant. In later studies, Harris and Switzer (78) demonstrated accelerated nasal clearance of *B. bronchiseptica* in swine subcutaneously vaccinated with either a sonicated *B. bronchiseptica* bacterin or a commercially prepared *B. pertussis* vaccine. This work stimulated researchers in Europe (13, 14, 125) to evaluate similar *B. bronchiseptica* whole-cell bacterins for use in the control of swine bordetellosis. Vaccination was reported to lower both the level of nasal turbinate atrophy and the infective level in a swine herd to a point where *B. bronchiseptica* infection no longer caused an economic problem. Additional studies have shown that vaccination reduces both the clinical signs and the nasal turbinate damage from atrophic rhinitis in infected swine herds (68), as well as in infected laboratory swine (42, 66). By vaccinating a population of young swine undergoing a bordetellosis epizootic, Goodnow (64) demonstrated that vaccination reduced clinical atrophic rhinitis by 90% and shortened the growth cycle by more than 3 weeks. In another study, vaccination improved the weight gain in weaning pigs (65).

Cross-immunity studies have verified that the *Bordetella* species possess common antigens. Evans and Maitland (40) prevented *B. bronchiseptica*-induced mortality and lung infections in intranasally challenged guinea pigs with a heat-killed suspension of *B. pertussis*. Later, Eldering (35) injected mice subcutaneously with thimerosal-inactivated whole cells of *B. pertussis* and demonstrated immunity in mice against infection and death after intraperitoneal inoculation of *B. bronchiseptica*. However, Winsser (165) failed to immunize mice intraperitoneally with a thimerosal-inactivated *B. pertussis* bacterin when they were inoculated intranasally with *B. bronchiseptica*.

There have been attempts to improve the immune response to swine bordetellosis by utilizing a subunit *B. bronchiseptica* vaccine. Normally, subunit vaccines are prepared to concentrate specific protective antigens or to remove

from microorganisms virulent or immunosuppressive factors which either act as poor immunogens or are unsafe for vaccine use (5, 120). Carlo et al. (D. J. Carlo, A. Hagopian, and P. J. Kniskern, U. S. patent 4,203,970, May 1980) prepared a subcellular *B. bronchiseptica* vaccine containing concentrated antigenic cell wall fraction. This vaccine provided significantly greater protection for *B. bronchiseptica*-infected swine against nasal turbinate histopathology than did a whole-cell *B. bronchiseptica* bacterin tested in similar fashion.

Predictably, the optimal method for controlling respiratory membrane infections is by the inducement of local immune factors. For this response the delivery and establishment of a specific immunogen at the site of infection are necessary. As with subunit vaccines, there have also been attempts to improve the control of *B. bronchiseptica* respiratory infections with live, intranasally delivered, avirulent *B. bronchiseptica* vaccines. Shimizu (141) first reported the isolation of a temperature-sensitive *B. bronchiseptica* mutant which, when delivered intranasally, provided high resistance in guinea pigs to *B. bronchiseptica*-induced hemorrhagic pneumonia. This work was followed by attempts to control canine bordetellosis with a similar live, avirulent vaccine. Goodnow and Shade (67, 139) vaccinated dogs intranasally, which then demonstrated resistance against clinical bordetellosis and hemorrhagic pneumonia, starting an average of 5 days postvaccination. Using a similar vaccine, Shade et al. (F. J. Shade, R. Bey, R. Goodnow, and R. Johnson, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, p. 65) showed a correlation between the occurrence of *B. bronchiseptica*-specific immunoglobulin A in the nasal secretions of intranasally vaccinated dogs and clinical resistance against canine bordetellosis.

Parenterally delivered, inactivated whole-cell bacterins, subunit *B. bronchiseptica* vaccines, and avirulent live *B. bronchiseptica* vaccines have been used to reduce clinical symptoms and histopathology and to accelerate clearance of *B. bronchiseptica* infections from the respiratory tracts of dogs, swine, and guinea pigs. However, the use of these vaccines has not led to a method for bordetellosis eradication. Methods for inducing total inhibition of either attachment or toxic effects on respiratory tract membranes by pathogenic *B. bronchiseptica* are still under development.

ECONOMIC EFFECTS

B. bronchiseptica infections of mammals, particularly swine and dogs, have caused the loss of millions of dollars from canine and swine pneumonia, disfigured swine, lost sales, drug costs, veterinary fees, and inefficient use of rearing and boarding facilities. Surveys indicate that 25 to 50% of the world swine population is infected and thus affected economically by *B. bronchiseptica* infections (152). Controlled vaccine efficacy field studies have shown that swine bordetellosis causes economic losses by stopping or lengthening the growth cycle of infected swine (65, 67, 68, 74). Furthermore, losses from swine destroyed due to disfigurement also occur (64). Shuman and Earl (144) demonstrated that sows in a *B. bronchiseptica*-infected herd produced significantly more stillborn pigs at farrowing and that the surviving piglets exhibited a significantly slower growth rate throughout the growth cycle, compared with normal pigs. Muirhead (114) evaluated the economics of an endemically *B. bronchiseptica* AR-infected 100-sow herd in England and found that the infection cost the producer approximately $9,850,00 annually from mortality, sacrificed pigs, medicated feed, veterinary fees, sales losses from deformed pigs, daily weight gain loss, and poor feed conversion. Although *B. bronchiseptica*-related infections are often cited as one of the top five disease problems of swine worldwide (114), the global economic impact from lost revenue attributed to *B. bronchiseptica* infections in commercial rearing businesses has not been well documented. Both government officials and animal producers hesitate to admit the presence of such respiratory infections. Animals with bordetellosis are often quarantined, preventing their export and import. As laboratory animal supply operations often depend upon selling respiratory pathogen-free animals, reports of the true incidence of and economic losses due to bordetellosis are also seldom cited. Similarly, canine breeders and commercial boarding kennels often minimize the occurrence of respiratory epizootics in their facilities. Approximately 11×10^6 dogs are boarded in kennels annually in the United States, and successful canine boarding is often dependent upon control of highly contagious canine respiratory infections.

CONCLUSIONS

Although *B. bronchiseptica* has been recognized as a respiratory pathogen of mammals since 1910 and although much of the basic biology of this organism has been described, numerous key pieces of information are still lacking. Further investigation is needed to determine the pathogenic mechanisms of *B. bronchiseptica* with respect to respiratory membrane attachment and alteration, the physical nature and biochemical nature of the endotoxins and heat-labile dermonecrotic toxins produced, and the possible relationship between pathogenicity and

the environmental conditions of the host (e.g., gaseous and particulate air pollutants). Furthermore, there is no universal acceptance concerning (i) the use of colonial phase typing for subspecies designation, (ii) the use of virulence and avirulence for isolate description, or (iii) the suggestion that *B. bronchiseptica* isolates carry ubiquitous protective antigens. A more meaningful language dealing with isolate similarities and differences should be developed. Finally, although numerous studies have demonstrated that *B. bronchiseptica* inactivated bacterins, subunit vaccines, and live intranasal vaccines can induce immunity in various mammals against clinical bordetellosis, eradication of the carrier state in vaccinated animals is yet to be demonstrated satisfactorily. Further work is needed to explain the immune mechanisms induced by *Bordetella* antigen.

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